

# Demand for Nonhuman Primate Resources in the Age of Biodefense

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## Abstract

The demand for nonhuman primates will undoubtedly increase to meet biomedical needs in this current age of biodefense. The availability of funding has increased the research on select agents and has created a requirement to validate results in relevant primate models. This review provides a description of current and potential biological threats that are likely to require nonhuman primates for the development of vaccines and therapeutics. Primates have been an invaluable resource in the dissection of viral disease pathogenesis as well as in testing vaccine efficacy. DNA vaccine approaches have been studied successfully for Ebola, Lassa, and anthrax in nonhuman primate models. Nonhuman primate research with monkeypox has provided insight into the role of cytokines in limiting disease severity. Biodefense research that has focused on select agents of bacterial origin has also benefited from nonhuman primate studies. Rhesus macaques have traditionally been the model of choice for anthrax research and have yielded successful findings in vaccine development. In plague research, African green monkeys have contributed to vaccine development. However, the disadvantages of current vaccines will undoubtedly require the generation of new vaccines, thus increasing the need for nonhuman primate research. Unfortunately, the current biosafety level (BSL)-3 and BSL-4 facilities equipped to perform this research are limited, which may ultimately impede progress in this era of biodefense.

**Key Words:** anthrax; arenaviruses; bioterrorism; bunyaviruses; filoviruses; nonhuman primates; plague; SARS

## Introduction

An increased demand for nonhuman primates will undoubtedly characterize the new era in which bioterrorism has become a reality. The US Food and Drug Administration (FDA<sup>1</sup>) has established new guidelines for

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<sup>1</sup>Abbreviations used in this article: BSL, biosafety level; EBOV, Ebola virus; FDA, Food and Drug Administration; LAV, Lassa arenavirus; LVS, live vaccine strain; MARV, Marburg virus; PA, protective antigen; SARS, severe acute respiratory syndrome.

testing vaccines and therapeutics of select agents—those pathogens that the government has determined are potential biological weapons. Because the actual risk of infection via a bioterrorist attack is not known, and because naturally occurring infections are quite rare, it is neither ethical nor practical to perform the usual phase 1, 2, and 3 trials. The ability to determine efficacy would be prohibitively expensive because most bioterror agents occur very rarely in nature and our ability to determine efficacy would take years. For this reason, FDA has ruled that stockpiles of vaccines and therapeutics can be generated if the treatment has been determined to be effective in two different animal models. Certainly at least one of the animal models should be a nonhuman primate. Below is a description of the select agents and other emerging pathogens that are likely to require the use of nonhuman primates.

## Viral Pathogens

### Filoviruses

Filoviruses are negative sense single-segmented RNA viruses. The most well known of the filoviruses is Ebola virus (EBOV<sup>1</sup>). Ebola was first discovered in Central Africa in 1976, and was named after a river in the Democratic Republic of the Congo (formerly Zaire). The outbreak began with the infection of individuals from a cotton factory, and it spread to relatives of those index cases (Feldmann et al. 2003). Ultimately, the total number of infected individuals was 284, with 151 deaths (Pattyn 1978). A second epidemic developed that year, and the case fatality rate was 88%.

EBOV emerged in the United States in 1989. The virus was detected in cynomolgus monkeys (*Macaca fascicularis*) that had been imported from the Philippines into a primate facility in Reston, Virginia (Jahrling et al. 1990). The virus appeared to spread through large droplets and/or small-particle aerosols. To date, the route of transmission has remained a unique feature that is associated with only this strain of Ebola, known as Ebola-Reston virus. It has not been associated with human disease, although it is classified as a biosafety level (BSL<sup>1</sup>)-4 agent.

Since the original description of EBOV, the virus has re-emerged several times in central Africa. It became a real threat to public health when it re-emerged in 1995 in Kikwit, Democratic Republic of the Congo. The current outbreak in the Congo has affected many great apes (Walsh et al. 2003).

EBOV and its relative Marburg virus (MARV<sup>1</sup>) have been actively used in weapon development programs, and they pose a potential bioterrorism threat (Alibek and Handelman 1999).

Nonhuman primates are the preferred animal model for the study of human filovirus infection because those animals are fatally infected with EBOV and MARV. Numerous species have been used, including baboons, African green monkeys, rhesus and cynomolgus macaques; and the pathology is similar to the infections observed in humans. It will be difficult to conduct vaccine clinical trials in humans for any EBOV vaccines because of the sporadic nature of the outbreaks and the potential ethical difficulty in obtaining approvals. The relatively rare appearance of EBOV does not favor normal development of a commercially viable vaccine. However, this view changes with the newer, existing threats of bioterrorism. If a vaccine became available, it would most likely be given to medical personnel and first responders, similar to those identified at risk from a smallpox attack. It has become clear that rodent models are not necessarily predictive for efficacy of antivirals and other therapeutics, so the gold standard will remain the nonhuman primate trials (Feldmann et al. 2003; Jahrling et al. 1996). In fact, in a vaccine efficacy study in nonhuman primates, it was demonstrated that vaccine strategies successful in rodents were not able to protect primates from a 1000-plaque forming unit challenge of virus (Geisbert et al. 2002).

## Arenaviruses

Arenaviruses are bisegmented single-stranded RNA viruses. Each segment is ambisense, and contains two convergent arranged genes separated by an intergenic hairpin loop. Among the several hemorrhagic fevers that are caused by arenaviruses are Lassa arenavirus (LAV<sup>1</sup>), Argentinian hemorrhagic fever (Junin virus), Bolivian hemorrhagic fever (Machupo virus), Venezuelan hemorrhagic fever (Guanarito virus), and Sabia virus. Lymphocytic choriomeningitis virus is the prototype of the family.

LAV is one of the most highly pathogenic arenaviruses. Named after the town of Lassa, in West Africa, the natural reservoir of this virus is the rodent species *Mastomys natalensis*. LAV has infected hundreds of thousands of people each year in West Africa. The yearly death toll totals approximately 10,000 (McCormick 1987). Symptoms of infection include fever and edema, with hemorrhage in fatal cases (Cummins et al. 1992). In some areas of Sierra Leone, the disease accounts for 30% of medical in-hospital deaths, 30% of deafness, and 70% of spontaneous abortion. Transmission from rodents to humans is believed to be by inhalation. Nosocomial spread of LAV also takes place in hospitals. Monkeys immunized with a less virulent related virus of Lassa, Mopeia, were protected (Fisher-Hoch et al. 1989) from Lassa infection. However, there is currently no approved vaccine for Lassa.

Junin virus has been identified in several rodent species

including *Calomys musculinus*. In north central Argentina, there is 12% seropositivity in humans. After an incubation period of 1 to 2 wk, nonspecific symptoms appear and are followed 1 wk later with more severe cardiovascular, renal, and neurological involvement. A live, attenuated Junin virus vaccine, Candid-1, has proven safe and effective in guinea pig and nonhuman primate trials (McKee et al. 1992, 1993). Clinical trials in human males have shown efficacy (Maiztegui et al. 1998).

The reservoir of Machupo virus is *Calomys callosus*. Both rhesus monkeys and African green monkeys demonstrate clinical signs consistent with the human disease (McLeod et al. 1976, 1978; Wagner et al. 1977). Guanarito virus is carried by *Zygodontomys brevicauda* (Fullhorst et al. 1997, 1999). Sabia was isolated in Brazil from a fatal human case. No nonhuman primate models of Guanarito or Sabia have been described to date (Whitton 2002).

## Bunyaviruses

Bunyaviruses are trisegmented negative sense RNA viruses. Hantaviruses are the causative agents of hemorrhagic fever with renal syndrome in Eurasia. Recently a novel hantavirus (Sin Nombre virus) was identified as the cause of hantavirus pulmonary syndrome, a potentially lethal condition first identified in 1993 in the southwestern United States. Hantaviruses have been found to be widespread throughout North and South America (Snell 2003). Andes virus was the first known hantavirus to show human-to-human spread; all of the other hantaviruses are rodent borne (Vitek et al. 1996). Because Andes virus has not been well studied, the probability of this agent being used as a biological weapon is unknown. Recently, nonhuman primates were used in attempts to understand the pathogenesis of the virus and the mechanisms of protection (McElroy et al. 2002). It was demonstrated that cynomolgous macaques, although not manifesting clinical disease, did manifest lymphocyte decrease during infection (McElroy et al. 2002). In addition, it was determined that antibodies generated in rhesus macaques against the Andes G1 and G2 protected Syrian hamsters from lethal challenge, which provided insight into potential postexposure treatment (Custer et al. 2003).

Crimean-Congo hemorrhagic fever was first observed in the Crimea in 1944 and 1945. Using human volunteers, it was determined that the agent is filterable and that the disease in humans is associated with tick bites. Congo virus was first isolated in Africa from the blood sample of a patient in 1956. In 1967, of 12 cases, five were identified as laboratory infection. The virus was later classified as a Nairovirus in the Bunyaviridae family. It can be transmitted nosocomially, and is a potential bioterrorist threat due to the lack of vaccines or therapeutics against the agent (Gear et al. 1982).

Rift Valley fever was first identified in Egypt in 1977. Its range has continued to expand, and the most recent outbreak was recorded on the Arabian Peninsula (CDC 2000).

Rhesus macaques and humans develop viremia and liver damage with elevated liver enzymes. Rhesus monkeys and humans can suffer more serious disease with hemorrhagic phenomena (Morrill and Peters 2003).

## Paramyxoviruses

Paramyxoviruses are single-stranded negative sense RNA viruses. Nipah virus was first isolated in 1999, when the virus crossed the species barrier from bats to pigs. The virus caused encephalitis in infected humans, with up to 40% mortality (Lam and Chua 2002). Currently, no prophylaxis or vaccine exists for Nipah. However, the antiviral ribavirin, which was used as an empirical therapy in infected patients, has been reported to be effective, although it has yet to be fully evaluated in animal experiments (Chong et al. 2001). There is a hamster animal model, in which animals die of acute encephalitis following Nipah virus infection (Guillaume et al. 2004; Wong et al. 2003). The model has shown that passive transfer of antibody from immunized animals protects them from a lethal Nipah virus challenge. In humans, both relapsing and late-onset cases of infection have been observed (Lim et al. 2003; Tan et al. 2002; Wong et al. 2001). In the situations described above, the immunobiology of the infection is unknown. None of these late pathologies have been observed in the hamster model (Guillaume et al. 2004). In Bangladesh, there is a current outbreak of Nipah (H. Feldmann, Canadian Center for Human and Animal Health, Winnipeg, Manitoba, personal communication, 2004), which has been shown to have nosocomial transmissibility (Tan and Tan 2001).

Nipah is a potential agent for bioterrorism based on several of its characteristics. (1) It can be produced in large quantities in cell culture, an important criterion for weaponization; (2) it has the potential for aerosol infection (Lam and Chua 2002); (3) outbreaks cause widespread panic and fear because of high mortality; (4) the highly virulent virus spreads easily among pigs and is easily transmitted to humans; (5) there is considerable social disruption and tremendous economic loss to an important pig-rearing industry; and (6) in addition to causing acute infection, it can cause clinical relapse months and years after infection.

## Other Emerging Viruses

### Severe Acute Respiratory Syndrome (SARS<sup>1</sup>)

With the emergence of SARS in 2003, the world has a very good model for the effect an outbreak of an unknown virus has on global health, international travel, and the economy of one or more regions. The SARS outbreak resulted in 8,098 cases, involving 774 deaths. Fear of the disease was great in many communities, especially among healthcare workers; and the billions of dollars lost in the airline and

tourism industries have resulted in bankruptcies of airlines and other businesses (Lingappa et al. 2004).

Although it is currently not listed as a select agent, recent cases of laboratory-acquired SARS may convince the US government that it certainly has potential as a biological weapon. Its mortality rate and ability to survive on surfaces long after other lipid-enveloped viruses become inactive make it a very good candidate to become a select agent in the near future. Currently a total of 12 candidate vaccines have been developed for SARS, and all of them require validation in nonhuman primates. The initial reports of SARS infection in cynomolgus macaques are proving to be controversial, and other animal models demonstrate variable clinical features at best. Current models appear to address viral replication in the absence of clinical reproducibility (Bisht et al. 2004; Fouchier et al. 2003; Martina et al. 2003).

## Monkeypox

The first human monkeypox cases in the United States were reported in May and June 2003 (CDC 2003a,b; Reed et al. 2004). Most of the individuals were believed to have acquired the infection from prairie dogs (*Cynomys* spp.) that became ill after contact with various exotic African rodents shipped from Ghana to the United States in 2003. Before 2003, monkeypox had been a health concern for human populations in equatorial Africa (Arita et al. 1985; Jezek et al. 1987, 1988). Research into the natural biology of monkeypox has been limited both because the disease is rare in humans and because no descriptions of naturally acquired animal infections exist (Guarner et al. 2004). Nonhuman primate research has provided valuable insight into the role of interferon in limiting disease severity (Cosgriff et al. 1989; Morrill et al. 1990, 1991). Further understanding of the pathogenesis of this disease as well as determination of the efficacy of vaccinations would benefit from expanded nonhuman primate research.

## Flaviviruses

Flaviviruses are positive sense, single-stranded RNA viruses. Although not a select agent or a bioterrorism threat, dengue virus is now endemic in much of South America and Asia, and vaccine and therapies vie for nonhuman primates resources. Although studies have been performed intensively for more than 50 yr on the development of a vaccine, there is still no commercial vaccine available against dengue disease. The lack of a suitable animal disease model has been detrimental to the development of a tetravalent live, attenuated dengue vaccine (Saluzzo 2003). Two candidate live, attenuated dengue vaccines currently exist. Chimeric and DNA vaccines also are in various stages of development.

The West Nile virus emerged in eastern North America

in 1999. It was a major concern even in modern arbovirology, not only because of its disease potential but also because it alerted the world that pathogens may turn up anywhere and at any time. Because West Nile virus is known to cause viremia in humans, blood transfusion is a potential risk. The first cases of transfusion transmission were documented in 2002 (Komar 2003). A number of candidate vaccines are in various stages of development, and similar dengue viruses will eventually require access to non-human primate resources.

## Bacterial Pathogens

### *Bacillus anthracis*

*B. anthracis*, the agent of anthrax, is a gram-positive bacterial pathogen that infects both humans and animals. Natural human infections occur when individuals handle materials from infected animals or inhale spores associated with animal products (Hanna 1998). Infection can occur in three forms: cutaneous, inhalation, and gastrointestinal. If left untreated, all forms of the disease can result in death (Meselson et al. 1994). The most fatal form of the disease, inhalation anthrax, presents with sore throat, mild fever, muscle aches, and malaise. The mortality rate is approximately 50%, even with care that includes the use of antibiotics (Inglesby et al. 2002). The recent use of this agent as a biological weapon has increased the importance of research focused on postexposure treatment (Inglesby et al. 1999, 2002).

The rapid onset of death in inhalation anthrax is due to septicemia and toxemia (Dixon et al. 1999). The bacterium secretes three proteins: edema factor, lethal factor, and protective antigen (PA<sup>1</sup>) (Elliott et al. 2000; Miller et al. 1999; Mourez et al. 2003; Wesche et al. 1998). Lethal toxin and edema toxin exert their effect when they bind the receptor-binding component (PA) of the A-B type toxin (Pezard et al. 1991). In fact, vaccination strategies have targeted PA in immune responses to prevent coupling of the effector toxin to the receptor-binding component (Welkos et al. 2001). PA has also been the focus of therapeutic recombinant antibodies that may eventually be used to treat cases of late-stage anthrax intoxication (Maynard et al. 2002).

Historically, the nonhuman primate model for inhalation anthrax has been the rhesus macaque (Friedlander et al. 1993; Fritz et al. 1995; Gleiser et al. 1963; Ivins et al. 1998; Lincoln et al. 1964). However, the increasing difficulty in obtaining animals for research has led to the need to develop another model that can sufficiently mimic human infection. Attempts have been made to establish rabbit models and other small animals for inhalation anthrax (Welkos et al. 2001; Zaucha et al. 1998), but nonhuman primate models continue to be needed for vaccine efficacy testing and other therapeutic testing. For instance, a survey of the efficacy of the anthrax vaccine adsorbed anthrax vaccine among guinea pigs, rabbits, and rhesus macaques resulted in variable pro-

tection when challenged with *B. anthracis* from diverse geographical origins (Fellows et al. 2001). Recent studies have shown that cynomolgus macaques exhibit pathology similar to that seen in humans and rhesus monkeys (Vasconcelos et al. 2003).

### *Francisella tularensis*

The gram-negative coccobacillus *F. tularensis* is the causative agent of tularemia. The former Soviet Union was widely reported to have developed *F. tularensis* as weaponry (Alibek and Handelman 1999). The organism was first isolated in 1911 in Tulare County, California, during an outbreak of a plague-like disease (Ellis et al. 2002). The natural reservoir includes small mammals such as ground squirrels, hares, voles, muskrats, water rats, rabbits, and other rodents (Grunow et al. 2000). This agent is spread to humans from infected reservoirs and other small vertebrates through direct contact or transmission from an arthropod vector. Tularemia is manifested in various ways, including ulcers at the site of inoculation, pharyngitis, lymphadenopathy, and pneumonia (Ellis et al. 2002). Immediate treatment with appropriate antibiotics can reduce the possibility of life-threatening pneumonia.

A live, attenuated vaccine was initially developed by the former Soviet Union in 1942 (Sjostedt et al. 1996) and subsequently transferred to the United States in 1956. Researchers identified two variants (blue and gray colony types) in the Soviet's vaccine strain. Immunization of guinea pigs with the blue variant resulted in increased resistance to lethal challenge (Eigelsbach and Downs 1961). Ultimately, the current live vaccine strain (LVS<sup>1</sup>) was derived from this variant, and its efficacy was tested in human volunteers (Saslaw et al. 1961). Because the basis of the LVS attenuation has not been determined, as well as lack of data regarding the characterization of the immune response, the vaccine is not currently available in the United States. Future vaccine development would benefit from the establishment of a nonhuman primate model.

During the initial phase of *F. tularensis* infection, a transient bacteremia occurs with dissemination of the pathogen throughout the host within the reticuloendothelial tissues. The presence of a capsule aids in establishing early bacteremia by shielding the organism from complement-mediated lysis (Sandstrom et al. 1988). In addition, phase variation appears to play a role in sustaining the viability of the organism within macrophages. Variation in LPS, specifically the O antigen and lipid A, reduces nitric oxide production and permits bacterial growth within macrophages (Cowley et al. 1996). A final known virulence determinant is an ABC transporter encoded on the *valA* gene, which is essential for macrophage growth (Cowley et al. 1996).

Despite the identification of several virulence factors, little is known about the overall pathogenicity or genetic make-up of *F. tularensis*. Animal models have been re-

stricted almost exclusively to murine models (Chen et al. 2003; Eigelsbach et al. 1975; Golovliov et al. 1995; Kudelina and Olsufiev 1980). These resources have been successful in dissecting early cytokine responses to tularemia. Nevertheless, nonhuman primate models may be needed to investigate pathogenesis (Alibek and Handelman 1999).

### *Yersinia pestis*

*Y. pestis* is a gram-negative bacillus that causes a disease historically referred to as “the plague.” It is primarily a zoonotic disease transmitted through the bite of an infected flea, and less commonly through the handling and consumption of infected animal tissue (Brubaker 1991). The agent causes several forms of the disease, namely bubonic, septicemic, and pneumonic plague. Bubonic plague is characterized by swollen tender lymph glands, fever, headache, and chills, and occurs when the bacteria are transmitted by an arthropod vector or when *Y. pestis* contaminated material enters the body through a break in the skin (Hull et al. 1987; von Reyn et al. 1977). Septicemic plague, which results when bacteria multiply in the blood, may be a complication of bubonic or pneumonic plague (Hull et al. 1987). In addition to the symptoms seen with bubonic plague, septicemic patients also present with abdominal pain, shock, and hemorrhaging into skin and internal organs (Hull et al. 1987). Finally, pneumonic plague is the contagious form of the disease that is spread through aerosolized bacteria (Doll et al. 1994; Meyer 1961). It is characterized by rapidly developing pneumonia, shortness of breath, cough and chest pain. Infected individuals must be treated within 24 hr to prevent a fatal outcome.

The highly pathogenic nature of *Y. pestis* is the result of multiple virulence factors. A major factor that prevents uptake of bacteria by immune scavenger cells is the F1 capsular antigen (Cornelis et al. 1998; Parkhill et al. 2001). *Y. pestis* cell wall components, V and W antigen, are lipoprotein complexes that play a role in preventing phagocytosis. The serum resistance observed in plague infections is the result of the short polysaccharide of *Y. pestis*, which prevents attachment of complement terminal attack complex (Persson et al. 1995). In addition, murine toxin has been shown to induce shock and respiratory distress in mice that is reminiscent of the human disease (Montie 1981).

Two vaccines are currently available. One vaccine is an attenuated strain of *Y. pestis*, and the other is a killed vaccine originally developed in the United States in the early 1940s (Meyer 1970; Williams et al. 1980). The vaccine is usually given only to those at risk for encountering a highly pathogenic strain, such as military troops and researchers. Despite the availability of a vaccine, there is interest in developing an improved vaccine because of major disadvantages with current vaccines. Previously, only a sparse number of studies have used African green monkeys and vervets in vaccine and pathogenesis studies of the plague

(Chen et al. 1976, 1977; Davis et al. 1996). As new vaccine candidates are identified, the need for a nonhuman primate model will certainly increase.

## Nonhuman Primate Resources

Nonhuman primates remain the best predictor of success for vaccines and therapies in humans. There is general agreement that the national primate centers could not undertake significant new research endeavors in any area, including biodefense, without significant expansion and investment. They all are currently working at capacity. Development of smaller nonhuman primates could be one way to enhance resources. Nevertheless, the nonhuman primate resources must be expanded and funded to satisfy the research needs in biodefense.

## Summary

The need for nonhuman primates is expected to grow enormously as vaccines and therapies against biological weapons and emerging diseases are developed. Smaller primates such as the marmoset may become very important for these studies, because space is very limited for primates in BSL-3 and -4 facilities. Unfortunately, although the amount of funding for biodefense-related research provided through regional centers of excellence and other funding sources at the National Institute of Allergy and Infectious Diseases has exponentially increased the interest in select agent research, only a small fraction has been provided to primate resources, and it is inadequate to deal with all of the vaccines and therapies being developed. Yet the development of nonhuman primate models continues to be critical to ensure that the true utility of infectious disease research will be of value to a population at risk of bioterrorism and infectious diseases.

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